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CASE REPORT

TOXICOLOGY

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Testing Antemortem Blood for Ethanol Concentration from a Blood Kit in a Refrigerator Fire

ABSTRACT: The stability of ethanol in antemortem blood stored under various conditions has been widely studied. Antemortem blood samples stored at refrigerated temperature, at room temperature, and at elevated temperatures tend to decrease in ethanol concentration with storage. It appears that the stability of ethanol in blood exposed to temperatures greater than 38°C has not been evaluated. The case presented here involves comparison of breath test results with subsequent analysis of blood drawn at the time of breath testing. However, the blood tubes were in a refrigerator fire followed by refrigerated storage for 5 months prior to analysis by headspace gas chromatography. The subject's breath was tested twice using an Intoxilyzer 8000. The subject's blood was tested in duplicate using an Agilent headspace gas chromatograph. The measured breath ethanol concentration was 0.103 g/210 L and 0.092 g/210 L. The measured blood ethanol concentration was 0.0932 g/dL for both samples analyzed. Although the mean blood test result was slightly lower than the mean breath test result, the mean breath test result was within the estimated uncertainty of the mean blood test result. Even under the extreme conditions of the blood kit being in a refrigerator fire, the measured blood ethanol content agreed well with the paired breath ethanol test.

KEYWORDS: blood alcohol, headspace, gas chromatography, blood alcohol stability, breath test, toxicology, blood ethanol

In Arizona, a person who operates a motor vehicle while under the influence of intoxicating liquor upon the request of a law enforcement officer shall be asked to take a chemical test of the person's blood, breath, urine, or other bodily substance for the purpose of determining alcohol concentration. The test or tests are chosen by the law enforcement agency. Failure to comply with such a request can result in a one-year suspension of the person's driver's license. The current driving under the influence (DUI) program in the City of Scottsdale, AZ, requires people suspected of DUI to submit to a breath test to determine breath ethanol content. In addition, blood samples are drawn, typically during the fifteen-minute deprivation period of the breath testing process. When the case is not resolved based on the breath test, the forensic laboratory will analyze the blood sample for ethanol concentration. This two-pronged testing approach has greatly reduced the number of blood ethanol tests required of the forensic lab. This approach also provides measurements of ethanol content using two different techniques for legal purposes.

Breath testing provides an immediate result, whereas testing blood usually involves a delay during which time the blood is stored prior to analysis. In DUI cases involving analysis of blood, defense arguments often include various factors that could affect the ethanol content in blood samples stored under different conditions and time periods. Therefore, it is important to understand how the storage of antemortem blood samples can affect their ethanol content. Common forensic storage conditions include refrigeration (4°C), sealed blood tubes, and the use of preservatives and anticoagulants. Studies of antemortem blood stored refrigerated have consistently shown small decreases in ethanol concentration if any change was measured (1–5). Frozen samples have also shown a decrease in ethanol content with storage (6). Under nonstandard forensic storage conditions, room temperature, and elevated temperature, ethanol concentration has been shown to decrease in antemortem blood samples (3,4,7–9).

Upon review of the literature, it appears that no studies exist regarding the stability of ethanol in blood for temperatures higher than 38°C. The blood kit in the case presented in this report was involved in a fire that occurred in a refrigerator in which officers impound blood kits for temporary storage until the blood kits are picked up by Property Technicians to transfer to the Property and Evidence Building (see Fig. 1).

Methods

Blood was collected from a subject suspected of DUI on July 12, 2019, into two 10-mL gray-top Vacutainer® tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate (Becton, Dickinson and Company, Franklin Lakes, NJ). The blood tubes were sealed inside a plastic clamshell box which was sealed inside a cardboard box. The blood kit was placed in a refrigerator for temporary storage. On Saturday, July 13, 2019, there was a fire in the refrigerator. Based on the Fire Department Incident Report, it is estimated that the fire lasted 15–

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FIG. 1—*Interior of the refrigerator in which the blood kit was stored. The refrigerator was equipped with a secondary metal door inside to secure the evidence deposited in the refrigerator.*

FIG. 2—*Exterior of the blood kit as received by the laboratory. The subject's name has been blurred in the image.*

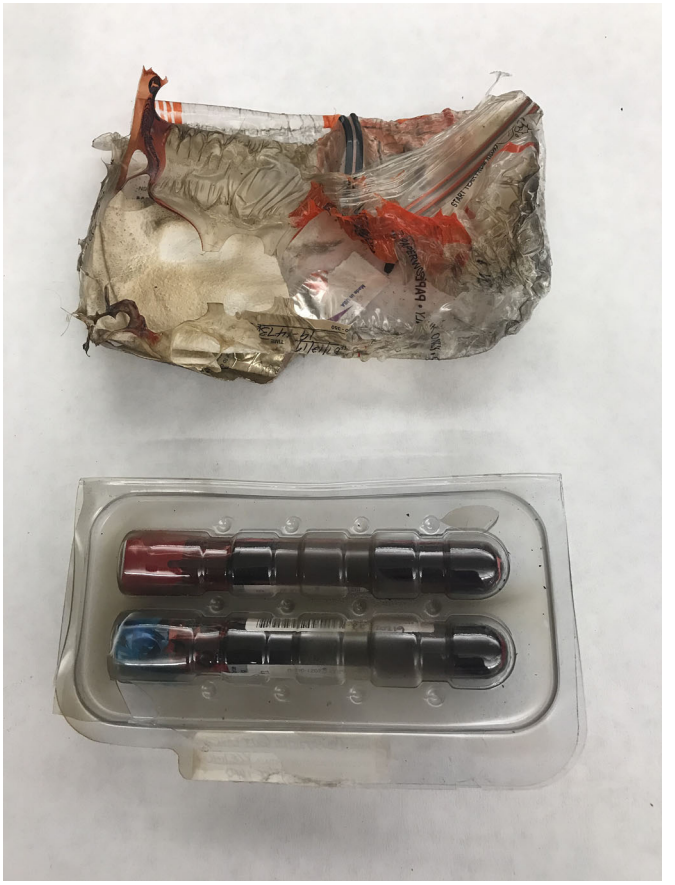

FIG. 3—*Interior of blood kit showing the melted bag and partial melting on the inner plastic box.*

20 min. As received by the laboratory, the outer box was partially burned (see Fig. 2). The inner plastic bag was melted to the cardboard box, and the inner plastic box had some melting on one end (see Fig. 3). The blood tubes were intact with no visible signs of damage. The blood was analyzed December 2, 2019, using an Agilent 7890B gas chromatograph connected to an Agilent 7697A Headspace Sampler (Agilent Technologies, Santa Clara, CA). The gas chromatograph was equipped with a dual-column system and two flame ionization detectors. The 30-m columns were the Agilent DB-ALC1 and DB-ALC2. The data from the DB-ALC1 column were used for the quantification of ethanol. The data from the DB-ALC2 column were used for confirmation of the identification of ethanol. Samples were incubated for 23 min at 60°C. Hydrogen was used as the carrier gas. The gas chromatograph held the oven temperature at 40°C for the analysis.

The gas chromatograph was calibrated using four calibrators with known ethanol concentrations: 0.02, 0.10, 0.20, and 0.40 g/dL. Following calibration and prior to cases samples being analyzed, the instrument was verified to be accurate using positive and negative controls. The case blood sample was tested in duplicate along with eleven other cases. Duplicate testing for each case consisted of one analyst preparing two separate samples from one blood tube and testing the two samples sequentially on the same instrument. Duplicate agreement was required to be within 2% of the mean of the two tests. A positive control was run between every five cases. Additional positive controls and a negative control were analyzed after all cases were analyzed.

A breath sample was collected and tested on July 12, 2019, in duplicate using an Intoxylizer 8000 (CMI, Inc., Owensboro, KY) following a fifteen-minute deprivation period during which the subject was watched to ensure that he did not belch or place anything into his mouth. The instrument was checked prior to the first test and after the second test using a 0.100 g/210 L dry

gas standard. An air blank was tested prior to and after each of the four tests. The two subject tests were taken at an interval not less than 5 min nor more than 10 min apart. The two subject tests were required to agree within 0.020 g/210 L.

Results and Discussion

The blood ethanol concentration was measured on the headspace gas chromatograph to be 0.0932 and 0.0932 g/dL. The uncertainty for blood ethanol measurement in our laboratory was calculated to be five percent at a level of confidence greater than 99.73 percent. The two results from the breath test were 0.103 g/210 L and 0.092 g/210 L.

There is good agreement between the breath test results and blood test results. On average, a blood result is expected to be higher than a breath result for a breath test instrument using a 1:2100 ratio (10–12). Acetaldehyde was also detected in the blood sample following analysis. Acetaldehyde may have been present due to the metabolism of ethanol in the subject. The presence of acetaldehyde has also been reported for blood samples heated in head space vials with a corresponding decrease in ethanol concentration (13). It is possible that the elevated temperatures in the refrigerator fire led to some reduction in ethanol concentration through oxidation of the ethanol to acetaldehyde. Additionally, the five months of refrigerated storage prior to analysis could also account for a slight decrease in ethanol concentration and corresponding increase in acetaldehyde.

Even under the extreme conditions of the blood kit being in a refrigerator fire, the measured blood ethanol content agreed well with the paired breath ethanol test.

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